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=> s methionine and hydrophobic and substitution and protein and plant  
L1 12 METHIONINE AND HYDROPHOBIC AND SUBSTITUTION AND PROTEIN AND  
PLANT

=> duplicate remove 11  
DUPLICATE PREFERENCE IS 'BIOSIS, EMBASE, CAPLUS'  
KEEP DUPLICATES FROM MORE THAN ONE FILE? Y/ (N) :n

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L2 11 DUPLICATE REMOVE L1 (1 DUPLICATE REMOVED)

=> d 12 1-11.ti

L2 ANSWER 1 OF 11 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.  
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TI Determinants of enzymatic specificity in the cys-met-metabolism  
PLP-dependent enzymes family: Crystal structure of cystathione  
 $\gamma$ -lyase from yeast and intrafamiliar structure comparison.

L2 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

TI Analysis of **plant**  $\alpha$ -tubulin with structural  
characteristics providing increased cold tolerance

L2 ANSWER 3 OF 11 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on  
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TI Engineered recombinant enteropeptidase catalytic subunit: Effect of  
N-terminal modification.

L2 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

TI The chaperone-like activity of a small heat shock **protein** is  
lost after sulfoxidation of conserved **methionines** in a  
surface-exposed amphipathic  $\alpha$ -helix

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TI A point mutation in a **plant** calmodulin is responsible for its  
inhibition of nitric-oxide synthase.

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TI High **methionine** derivatives of  $\alpha$ -hordothionin for  
pathogen-control

L2 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

TI High-**methionine** derivatives of  $\alpha$ -hordothionin and the  
transformation of improved **plant** crops

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TI Extensive modifications for **methionine** enhancement in the  
 $\beta$ -barrels do not alter the structural stability of the bean seed  
storage **protein** phaseolin.

L2 ANSWER 9 OF 11 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on  
STN  
TI Strategies for selecting mutation sites for **methionine**  
enhancement in the bean seed storage **protein** phaseolin.

L2 ANSWER 10 OF 11 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on  
STN  
TI BINDING OF THE YEAST TRNA-MET ANTICODON BY THE COGNATE METHIONYL-TRNA  
SYNTHETASE INVOLVES AT LEAST TWO INDEPENDENT PEPTIDE REGIONS.

L2 ANSWER 11 OF 11 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on  
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TI ISOLATION AND CHARACTERIZATION OF QCR9 A NUCLEAR GENE ENCODING THE 7.3-KDA  
SUBUNIT 9 OF THE SACCHAROMYCES-CEREVISIAE UBIQUINOL CYTOCHROME C  
OXIDOREDUCTASE COMPLEX AN INTRON-CONTAINING GENE WITH A CONSERVED SEQUENCE  
OCCURRING IN THE INTRON OF COX4.

=> d 12 9 ibib ab

L2 ANSWER 9 OF 11 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on  
STN  
ACCESSION NUMBER: 1994:82523 BIOSIS  
DOCUMENT NUMBER: PREV199497095523  
TITLE: Strategies for selecting mutation sites for  
**methionine** enhancement in the bean seed storage  
**protein** phaseolin.  
AUTHOR(S): Dyer, John M.; Nelson, Jeffrey W.; Murai, Norimoto [Reprint  
author]  
CORPORATE SOURCE: Dep. Biochemistry, Louisiana State Univ., Baton Rouge, LA  
70803, USA  
SOURCE: Journal of Protein Chemistry, (1993) Vol. 12, No. 5, pp.  
545-560.  
CODEN: JPCHD2. ISSN: 0277-8033.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 22 Feb 1994  
Last Updated on STN: 23 Feb 1994  
AB The complete three-dimensional structure of the bean seed storage  
**protein** phaseolin was generated from alpha-carbon coordinates by  
using molecular mechanic calculations. This structure was used as a  
template to simulate modifications aimed at increasing the  
**methionine** content of phaseolin. A hydrophilic,  
**methionine**-rich looping insert sequence was designed. Simulated  
mutagenesis shows that the insert might be accommodated in turn and loop  
regions of the **protein**, but not within an alpha-helix.  
**Methionine** content was also increased by the replacement of  
**hydrophobic** amino acids with **methionine** in the central  
core beta-barrels of the phaseolin **protein**. Calculations  
indicated that **methionine** can effectively replace conserved or  
variant leucine, isoleucine, and valine residues. However, alanine  
residues were much more sensitive to **substitution**, and  
demonstrated high variability in the effects of **methionine**  
replacement. Introduction of multiple **substitutions** in the  
barrel interior demonstrated that the replaced residues could interact  
favorably to relieve local perturbations caused by individual  
**substitutions**. Molecular dynamics simulations were also utilized  
to study the structural organization of phaseolin. The calculations  
indicate that there are extensive packing interactions between the major  
domains of phaseolin, which have important implications for  
**protein** folding and stability. Since the proposed mutant  
**proteins** can be produced and studied, the results presented here  
provide an ideal test to determine if there is a correlation between the

effects obtained by computer simulation and the effects of the mutations on the **protein** structure expressed in vivo.

=> d 12 8 ibib ab

L2 ANSWER 8 OF 11 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.  
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ACCESSION NUMBER: 96001948 EMBASE

DOCUMENT NUMBER: 1996001948

TITLE: Extensive modifications for **methionine**  
enhancement in the  $\beta$ -barrels do not alter the  
structural stability of the bean seed storage  
**protein** phaseolin.

AUTHOR: Dyer J.M.; Nelson J.W.; Murai N.

CORPORATE SOURCE: Department of Biochemistry, Louisiana State  
University, Baton Rouge, LA 70803, United States

SOURCE: Journal of Protein Chemistry, (1995) 14/8 (665-678).  
ISSN: 0277-8033 CODEN: JPCHD2

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 029 Clinical Biochemistry

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Common beans are widely utilized as a food source, yet are low in the essential amino acid **methionine**. As an initial step to overcome this defect the **methionine** content of the primary bean seed storage **protein** phaseolin was increased by replacing 20 evolutionarily variant **hydrophobic** residues with **methionine** and inserting short, **methionine**-rich sequences into turn and loop regions of the **protein** structure. **Methionine** enhancement ranged from 5 to 30 residues. An *Escherichia coli* expression system was developed to characterize the structural stability of the mutant **proteins**. **Proteins** of expected sizes were obtained for all constructs except for negative controls, which were rapidly degraded in *E. coli*. Thermal denaturation of the purified **proteins** demonstrated that both wild-type and mutant phaseolin **proteins** denatured reversibly at approximately 61°C. In addition, urea denaturation experiments of the wild-type and a mutant **protein** (with 30 additional **methionines**) confirmed that the structural stability of the **proteins** was very similar. Remarkably, these results indicate that the phaseolin **protein** tolerates extensive modifications, including 20 **substitutions** and two loop inserts for **methionine** enhancement in the  $\beta$ -barrel and loop structures, with extremely small effects on **protein** stability.

commands which can be used in this file.

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ENTER A FILE NAME OR (IGNORE):biosis

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FULL ESTIMATED COST	0.21	0.21

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=> s vsp and plant

L1 268 VSP AND PLANT

=> duplicate remove l1

DUPLICATE PREFERENCE IS 'AGRICOLA, BIOSIS, EMBASE, CAPLUS'

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PROCESSING COMPLETED FOR L1

L2 146 DUPLICATE REMOVE L1 (122 DUPLICATES REMOVED)

=> s l2 and substitution

L3 2 L2 AND SUBSTITUTION

=> d l3 1-2 ibib ab

L3 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2004:836369 CAPLUS

TITLE: A single amino acid **substitution** in soybean **VSP $\alpha$**  increases its acid phosphatase activity nearly 20-fold

AUTHOR(S): Leelapon, Oranuch; Sarath, Gautam; Staswick, Paul E.

CORPORATE SOURCE: Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE 68583, USA

SOURCE: Planta (2004), 219(6), 1071-1079

CODEN: PLANAB; ISSN: 0032-0935

PUBLISHER: Springer GmbH

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Soybean [Glycine max (L.) Merr.] contains two proteins called vegetative storage proteins (**VSPs**) that function as temporary storage reserves, but are also closely related to **plant** acid phosphatases of the haloacid dehalogenase (HAD) superfamily. This study examined the biochem. basis for the relatively low catalytic activity previously reported for these **VSPs**. The specific activity of purified recombinant **VSP $\alpha$**  on GMP was about 40-fold lower than for a related soybean root nodule acid phosphatase (APase), which had a specific activity of 845 U mg<sup>-1</sup> protein. Conversion of Ser106 to Asp increased **VSP $\alpha$**  activity about 20-fold. This Asp residue is present in nodule APase and is a highly conserved nucleophile in the

HAD superfamily. Related **VSPs** from cultivated soybean and from three wild perennial soybeans, as well as a pod storage protein (PSP) from *Phaseolus vulgaris* L. all lack the catalytic Asp, suggesting they too are catalytically inefficient. Phylogenetic anal. showed the **VSPs** and PSP are more closely related to each other than to 21 other **VSP**-like proteins from several **plant** species, all of which have the nucleophilic Asp. This study suggests that loss of catalytic activity may be a requirement for the **VSPs** and PSP to function as storage proteins in legumes.

L3 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1999:388313 CAPLUS

DOCUMENT NUMBER: 131:40549

TITLE: The use of protein engineering method to alter the amino acid content of vegetative storage protein (**VSP**) and increase its nutritional value

INVENTOR(S): Rao, Gururaj A.; Sleister, Heidi Major

PATENT ASSIGNEE(S): Pioneer Hi-Bred International, Inc., USA

SOURCE: PCT Int. Appl., 42 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9929882	A2	19990617	WO 1998-US26209	19981210
WO 9929882	A3	19990916		
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DK, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
ZA 9811327	A	19990614	ZA 1998-11327	19981210
AU 9939083	A1	19990628	AU 1999-39083	19981210
PRIORITY APPLN. INFO.:			US 1997-988015	A1 19971210
			WO 1998-US26209	W 19981210

AB Methods for altering amino acid composition of **VSP** are provided, particularly to proteins whose three-dimensional structure is unknown. The method comprises creating interacting mols. to the native protein and selecting for engineered proteins which retain the native conformation by antibody binding assay. In this manner, the levels of essential amino acids in a protein can be increaseed yet the biol. activity of the protein maintained. Three **VSP** variants have been proposed and the methionine of proteins can be increaseed about 10-20%, more preferentially about 20-40% by using this method.